BACTERIAL GROWTH WITHOUT NET PROTEIN SYNTHESIS

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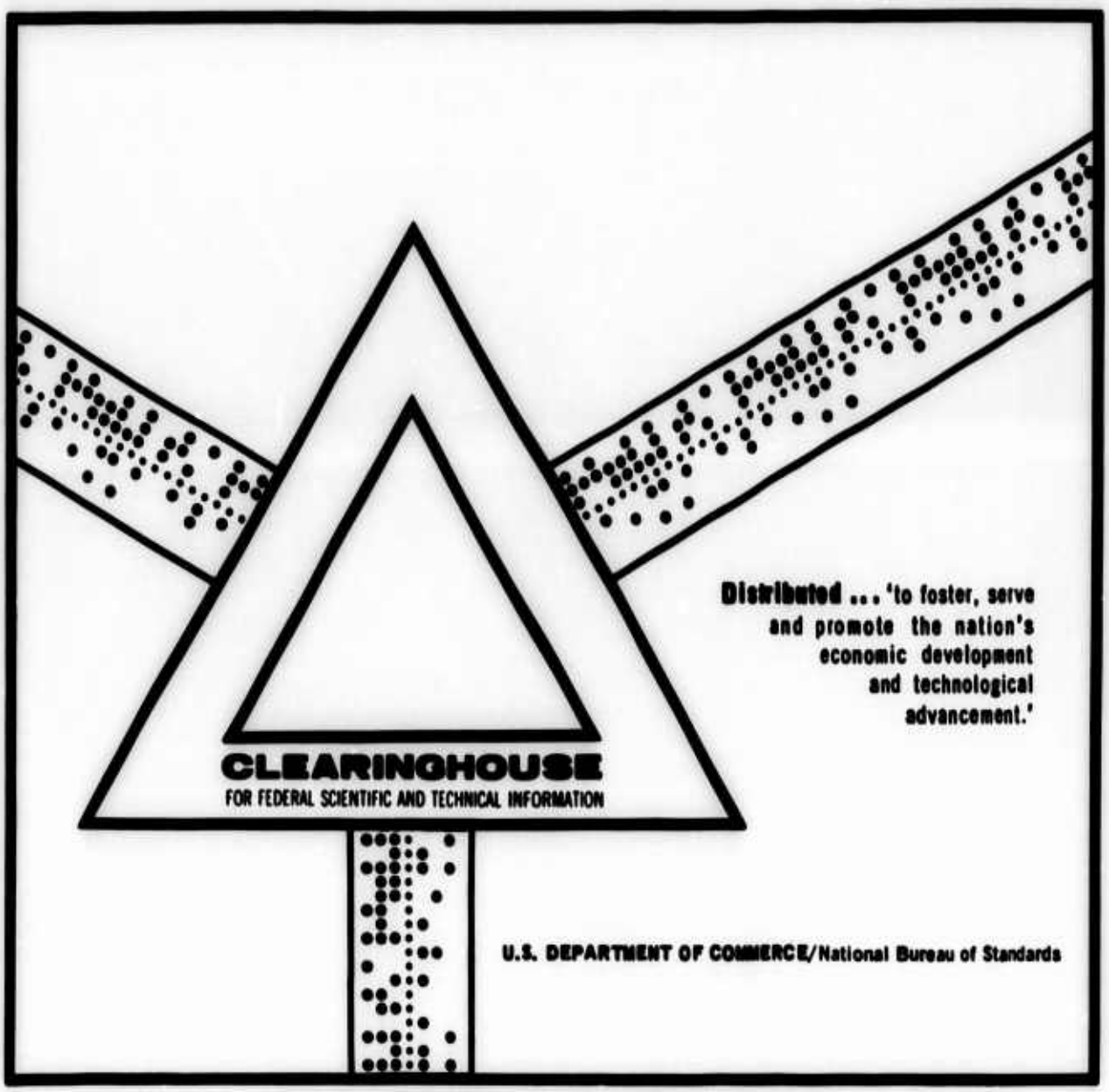

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FINAL REPORT

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This project has been concerned with postesponential growth and the so-called stationary growth phase of bacteria. Exponential growth can cease for different reasons, one of which is depletion of an essential nutrient from the medium. We have shown earlier that depletion of different nutrients results in different patterns of postexponential growth, and different types of "resting" cells (*S. faecalis*). These cell types can differ greatly in their percentage dry weight content of wall, membrane and cytoplasmic substance, and show corresponding morphological differences. The aim of the work under this Contract has been to understand the biochemical mechanisms which underlie these phenomena of morphological differentiation.

Concurrently with the ONR Contract the work has also enjoyed the support of the American Cancer Society and the National Institutes of Health. Additional work was made possible by an award from the Deutsche Forschungsgemeinschaft for a one-year guest professorship in Germany at the University of Göttingen and the Biologische Bundesanstalt in Braunschweig (1968-69).

During the contract period cystine has been the center of attention, because it had become apparent that this amino acid plays a major role in the synthesis of both wall and membrane substance during postexponential growth. During exponential growth the organism does not depend on exogenous cystine, as long as methionine is available, because cystine can be synthesized from methionine through a well-known metabolic sequence. However, under postexponential conditions optimal production of wall and membrane substance requires a nutritional supply of both methionine and cystine, i.e. methionine does not function as a precursor for cystine, and the question arises: Why?

In this connection it became clear that cystine, in addition to its better known major functions (source of -SH groups and -S-S- bonds) is also unique as an amino acid essential for the synthesis of the metabolic transfer agents, Coenzyme A and Acyl Carrier Protein (ACP). Both contain pantotheine, which originates metabolically from the vitamin pantothenate and the amino acid cystine, and both (CoA and ACP) are specifically needed in the metabolic transfer of acyl groups.

We know of two specific sites of acyl transfer in the biosynthetic events that underlie the growth of S. faecalis and similar organisms. These are (a) the acetylation of hexosamine in the synthesis of acetylmuramic acid, and (b) the acylation of fatty acids in the synthesis of lipids. Process (a)

appears to be specific for the synthesis of wall substance and process (b) for the synthesis of membrane substance, i.e. muramate is a specific constituent of wall and lipid is a specific constituent of membrane. The fact that the acyl carrier substances depend on cystine, and that they function specifically in connection with wall and membrane substance, thus seems to "explain" the role of cystine in connection with postexponential wall and membrane formation.

The evidence, mentioned above, that during postexponential growth cystine cannot be synthesized from methionine, may have its explanation in the metabolic instability of relevant enzymes and cofactors. This view is supported by several observations. First, the growth curve in a cystine-free medium closely resembles that in a cystine-containing medium during the initial 2 or 3 hours of postexponential culturing, but subsequently a sharp divergence develops, suggesting that apoenzymatic protein(s) concerned with the methionine-to-cystine reaction path, which are synthesized during exponential growth, "survive" postexponentially in sufficient amount for a limited time, until their metabolic degradation is completed, because of the lack of essential amino acid(s). Second, during exponential growth CoA undergoes rapid degradation and de novo synthesis. Third, indirect evidence suggests that this synthesis is severely restricted during postexponential growth, even though the essential precursors, including cystine and pantothenate, are available. Finally, in the experiments just

mentioned, the only truly essential amino acid present during postexponential growth was methionine. However, when all essential amino acids except one (threonine) are present postexponentially, the evidence suggests that also here the methionine-to-cystine process is severely restricted postexponentially, again implying enzyme degradation.

Some of these observations have not reached the stage of maturity for publication, because of lack of time. The publications which have matured during the contract period are listed below.

PUBLICATIONS

1. Toennies, G.and Joseph J. Kolb. Carbohydrate Analysis of Bacterial Substances by a New Anthrone Procedure. Anal. Biochem. 8, 54-69 (1964)

2. Toennies, G. and Frances Feng. Measurement and Characterization of Proteins by Color Reactions. Anal. Biochem. 11, 400-17 (1965)

3. Toennies, G., Frances Feng, Joseph J. Kolb and Phoebe M. Luttner. Bacterial Nucleate and Phosphorus Partition. Anal. Biochem. 11, 473-96 (1965)

4. Toennies, G. Role of Amino Acids in Postexponential Growth. J. Bact. 90 No.2, 438-42 (1965)

5. Toennies, G., D. N. Das and F. Feng. Pantothenate and Coenzyme A in Bacterial Growth. J. Bact. 92 No. 3, 707-13 (1966)

6. Toennies, G, D. N. Das and F. Feng. New Observations on the Determination of Bacterial Lipid Phosphorus. Canadian J. Microbiology, 14, 484-5 (1968)

7. Das, D. N. and G. Toennies. A Versatile Buffered Scintillation System. Anal. Biochem. 23, 26-34 (1968)

8. Das, D. N. and G. Toennies Relations Between Coenzyme A and Presumptive Acyl Carrier Protein in Different Conditions of Streptococcal Growth. J. Bact. 98 No. 3, 898-902 (1969)